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EFFECTS OF TEMPERATURE AND RELATIVE HUMIDITY ON THE SURVIVAL OF NEWCASTLE DISEASE VIRUS AEROSOLS IN THE ROTATING DRUM (U)

by

B. Kournikakis, D. Netolitzky* and J. Fildes

Project No. 351SH

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* Summer Research Assistant — May – August 1987

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ABSTRACT

The survival of aerosolized Newcastle Disease Virus (LaSota strain) was examined under 15 conditions of temperature and relative humidity (10°, 15°, 20°, 25°, and 30° C, each at low (20-30%), medium (50%) and high (80%) relative humidities (RH)) in the rotating drum system. Virus survival was best at 10° and at low RH.

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INTRODUCTION

Viruses constitute the single largest group of potential BW agents. Although a BW attack would most likely be effected through aerosol dissemination of agent, our knowledge of virus

aerosol stability in open air is minimal. In 1979, to assist in overcoming this deficiency, Canada initiated a program to develop a vertebrate virus BW simulant system to provide a safe method to study the characteristics and survival of virus aerosols in the open air. The model would also provide a model for the field testing of virus collection, detection, and identification techniques and for testing collective and individual protective equipment. This field testing is essential for the continued maintenance and improvement of the Canadian Forces ability to detect and apply countermeasures to deal with a biological attack.

Mofford and Fulton (14) reviewed several candidate non-pathogenic viruses and selected a commercially available poultry vaccine, the LaSota strain of Newcastle Disease Virus (NDV), as the virus simulant of choice. This virus is commonly used by poultry farmers in Canada to vaccinate their flocks and is not considered hazardous to humans or to animals in the environment.

NDV is one of the paramyxoviruses, a group which includes viruses such as parainfluenza and mumps. The virus is enveloped, 125 to 250 nm in size with an 18 nm nucleocapsid, and contains a single negative strand RNA molecule. It has been shown (15,17,18) that the virulence of Newcastle Disease Virus is directly related to its ability to form plaques

in cell culture monolayers. Since this avirulent vaccine strain of NDV will not normally form plaques in cell culture it was necessary to develop a modified plaque assay (9,10) in order to quantitate the amount of viable virus in experimental samples .

Aerosolization in open air subjects a virus to a number of factors (eg. temperature, relative humidity (RH), irradiation, free radicals, air ions, pollutants and "open air factor" (1)) which can affect the decay of virus viability. In addition, the process of aerosolization itself may induce losses in viability depending on the type of aerosol generator used. In order to better understand how some of these factors may interact in open air, it is necessary to study them under defined conditions. In the present report, we describe the effects of five temperature and three RH levels on the viability of aerosolized NDV contained within a rotating drum apparatus.

Materials and Methods

Cells and Virus

A continuous line of rhesus monkey kidney cells ($LLC\text{-}MK_2$) obtained from the American Type Culture Collection (Rockville, Maryland, USA) was grown as monolayers in Corning 150 cm^2 tissue

culture flasks (Corning Glassworks, Corning, N.Y., USA) with Earle's Minimum Essential Medium (MEM) (Flow Laboratories Inc., Missauga, Ontario) as described previously (9,10).

NDV vaccine (LaSota strain) (Salsbury Laboratories Ltd., Kitchener, Ontario) was obtained in lyophilized form and stored at 4^{o} C.

Reagents

Phosphate buffered saline (PBS) (Oxoid Canada, Ottawa, Ontario) was prepared in triple glass distilled water and sterilized by autoclaving. Complete PBS also contained 0.01% (w/v) $CaCl_2$ and 0.01% (w/v) $MgCl_2$.

Determination of Physical Decay

The use of a flourescent dye (sodium fluorescein) as a physical tracer provides an accurate method of determining the amount of virus lost to physical decay and to dilution of the virus aerosol within the drum (7). Excitation and emission wavelengths of 490 and 510 nm were used in estimating fluorescein tracer levels on a Kontron spectrofluorometer SFM 23/B (Tegimenta AG, Switzerland).

Aerosol generation, storage, and collection

Virus aerosols were generated using a 3-jet Collison nebulizer (11). The aerosol passed into a mixing tube where it was blended into a 250 L/min airflow equilibrated for the desired temperature and humidity. Control of temperature and RH in the airflow was effected electronically by a Honeywell Micronik 100 system (Honeywell Ltd., Calgary, Alberta). Temperature and RH during the experiment were monitored using wet/dry bulb thermometers incorporated into the drum system. The diluted aerosol then passed into a 500 L drum (5) rotating at a speed of 4 rpm to reduce sedimentation losses. Drum temperature was maintained by controlling the ambient temperature of the room. Samples of virus aerosol were collected through a sampling port into an all glass impinger (AGI-30)(20), with a flowrate of 11 L/min regulated by a Matheson Mass Flow controller Model 8200 (Matheson Gas Products Canada, Edmonton, Alberta). After each experiment the drum was flushed with flowing air for 15 to 30 min to remove any remaining virus and fluorescein tracer.

Test Procedure

Lyophilized NDV vaccine was reconstituted with PBS (4.5 mL per 1000 dose vial) and mixed with sterile 2% (w/v) sodium flourescein (Sigma Chemical Co., St. Louis, MO, USA)in PBS to a 0.2% final concentration of dye. The plaque titer of reconstituted vaccine was normally between 7 - 9 x 10^7 plaque forming units (pfu)/mL. The collison nebulizer, containing 10 mL of the virus/tracer mix, was attached to the mixing tube inlet of the drum and pressurized with 1.8 kg/cm^2 of air to aerosolize the virus. Virus was aerosolized for 8 min, after which the drum was isolated from the airflow by means of a valve, and the first sample was taken 15 min following the initial pressurization of the collison nebulizer. An AGI-30, containing 10 mL of complete PBS as the collection fluid, was operated for 2 min to collect 22 L of air. During this time the inlet valve to the drum was opened to allow 22 L of makeup air to flow into the drum to maintain normal pressure. The same procedure was used to collect samples hourly from 1 to 6 hr. The collected samples were kept in a refrigerator at 4^{o} C until they could be processed after the completion of the trial. The collected samples were dispensed into small test tubes and frozen at -70^{o} C for plaque assay at a

later date. Serial dilutions of one aliquot were prepared immediately to estimate the amount of physical tracer in each sample.

Calculations

The biological decay of the virus was estimated by using the following formula:

(Formula 1) $\%\ \text{Virus Survival} = (T_0/T_t) \times (V_t/V_0) \times 100$

where T_0 and T_t are the dye concentrations at times 0 and t respectively, and V_0 and V_t are the virus titers at times 0 and t. Time 0 in this case is the initial sample taken 15 minutes following the initiation of virus aerosolization.

The physical decay of the fluorescein tracer was calculated by the following formula:

(Formula 2) $\%\ \text{Physical Decay} = (T_t/T_0)/((DV - CSV_t)/DV) \times 100$

where DV is the total drum volume, and CSV_t is the cumulative sampling volume at time t.

Biological and physical decay data were fitted to an exponential decay function:

(Formula 3) $$V_t/V_0 = 100 \times e^{-k(t-0.25)}$$

where k represents a time constant (hr^{-1}). Biological and physical half life values were calculated by the following formula:

(Formula 4) $$t_{1/2} = \log_e(2)/k = 0.693/k$$

Two to four experiments were carried out for each temperature and RH setting (44 total). The settings used were 10^{o}, 15^{o}, 20^{o}, 25^{o} and 30^{o} C) at low, medium and high humidities. Due to limitations of the drum system, RH at the 2 cooler temperatures could not be adjusted lower than 30% RH. Low RH for the remaining three temperatures was set at 20%. Medium and high RH were set at 50% and 80%, respectively.

Results and Discussion

In developing a virus tracer system that would ultimately be used in the field under a variety of environmental conditions, it is essential to have some understanding of the effects of these environmental factors under defined conditions. Temperature and humidity are two important environmental factors that affect virus aerosols. In this study, we have examined aerosolized NDV

under 15 conditions of controlled temperature and RH to determine the effects of these conditions on the physical and biological decay of the virus aerosol.

Physical decay data of the virus aerosol in the drum (Table 1) was fitted to an exponential decay function (Formula 3) with a correlation coefficient >0.98 in all cases. Physical half lives, calculated and summarized in a three dimensional style graph (Fig. 1), show that the physical decay is variable over the different conditions of temperature and RH, but with no appreciable pattern to the variation.

Biological decay data for the virus aerosol tabulated in Table 2, was fitted to the exponential decay function with good correlation in 13 of the 15 cases (R^2 >0.94). In the two remaining cases (30°, medium RH, 30°, high RH), the correlation coefficient was lower (R^2 =0.700 and 0.819 respectively) but still significant. The physical half lives, calculated and summarized in a three dimensional style graph (Fig. 2), show a clear pattern of optimal virus survival at both low RH and at 10° C. At medium and high RH, virus survival decreases with increasing temperature.

As noted in a review by Sattar and Ijaz (16), it is generally believed that lipid containing viruses such as NDV survive better in conditions of low RH when aerosolized. Studies of aerosolized Newcastle Disease Virus (19) and other

parainfluenza viruses (3,12) all resulted in optimal virus survival at low RH. Studies of the effects of temperature on virus aerosols have shown that cooler temperatures can enhance virus survival (2,3,4,6,8,13). Both of these observations are in agreement with our own results with maximum virus survival occurring at 10° C and low RH.

Our results suggest that, for the purposes of field studies, temperature and RH alone may have a minimal effect on virus survival. In a field experiment, the period of time in which the virus would be in the aerosol state, as it travels downwind from the aerosol generator to the sampling device, is called the "transit time". The transit time of NDV aerosolized in the field would fall far short of the time required to inactivate much of the virus. In a "worst case" scenario, if a field experiment was conducted at 30° C and 50% RH (Biological half life = 0.72 hours) and the virus dispersed into a 10 km/hr wind and then sampled 1 km downwind, the transit time of the virus aerosol would be 0.1 hours (6 minutes). This short transit time would allow most of the virus to survive the effects of temperature and RH alone. Other factors (irradiation, open air factor, etc) during the field experiment would also be acting on the aerosol and could play a greater role in reducing the viability of the virus.

Summary

The non-pathogenic LaSota strain of Newcastle Disease Virus was aerosolized into a rotating drum system to determine the effects of temperature and RH on the survival of virus over a period of 6 hr. Low RH (<30%) and cool temperatures (10° C) appear to be the optimal conditions for survival of NDV in this system.

Since transit times of NDV aerosols in the field will likely be much shorter than the biological half lives observed in the drum system, our results suggest that RH and temperature should not play a major role in virus inactivation during field experimentation.

References

1. Cox, C.S. The Aerobiological Pathway of Microorganisms. Chap. 10-13, John Wiley & Sons Ltd., New York, 1987.

2. Elazahary, M.A.S.Y., and J.B. Derbyshire. Effect of temperature, relative humidity and medium on the aerosol stability of Infectious Bovine Rhinotracheitis Virus. Can. J. Comp. Med. 43:158-167, 1979.

3. Elazahary, M.A.S.Y., and J.B. Derbyshire. Aerosol stability of Bovine Parainfluenza Type 3 Virus. Can. J. Comp. Med. 43:295-304, 1979.

4. Elazahary, M.A.S.Y., and J.B. Derbyshire. Aerosol stability of Bovine Adenovirus Type 3. Can. J. Comp. Med. 43:305-312, 1979.

5. Goldberg, L.J., M.S. Watkins, E.E. Boerke, and M.A. Chatigy. The use of a rotating drum for the study of aerosols over extended periods of time. Am. J. Hyg. 68:85-93, 1958.

6. Ijaz, M.K., A.H. Brunner, S.A. Sattar, R.C. Nair and C.M. Johnson-Lussenburg. Survival characteristics of airborne human Coronavirus 229E. J. Gen. Virol. 66:2743-2748, 1985.

7. Ijaz, M.K., Y.G. Karim, S.A. Sattar and C.M. Johnson-Lussenburg. Development of methods to study the survival of airborne viruses. J. Virol. Methods 18:87-106, 1987.

8. Ijaz, M.K., S.A. Sattar, C.M. Johnson-Lussenburg and V.S. Springthorpe. Effect of relative humidity, atmospheric temperature, and suspending medium on the airborne survival of human Rotavirus. Can. J. Microbiol. 31:681-685, 1985.

9. Kournikakis, B., and J. Fildes. Development of a plaque assay for Newcastle Disease Virus. (U) Suffield Memorandum No. 1185, 1987. Unclassified

10. Kournikakis, B. and J. Fildes. Titration of avirulent Newcastle Disease Virus by the plaque assay method. J. Virol. Methods 20:285-293, 1988.

11. May, K.R. The collison nebulizer: description, performance and application. Aerosol Science 4:235-243, 1973.

12. Miller, W.S., and M.S. Artenstein. Aerosol stability of three acute respiratory disease viruses. Proc. Soc. Exp. Biol. Med. 125:222-227, 1967.

13. Moe, K., and G.J. Harper. The effect of relative humidity and temperature on the survival of Bovine Rotavirus in aerosol. Arch. Virol. 76:211-216, 1983.

14. Mofford, L., and R.E. Fulton. Review and evaluation of candidate virus tracers for use in field trials. (U) Suffield Report No. 313, 1982. Unclassified

15. Reeve, P., and G. Poste. Studies on the cytopathogenicity of Newcastle Disease Virus: Relation between virulence, polykaryocytosis and plaque size. J. Gen. Virol. 11:17-24, 1971.

16. Sattar, S.A., and M.K. Ijaz. Spread of viral infections by aerosols. CRC Critical Reviews in Environmental Control 17:89-131, 1987.

17. Schloer, G., and R.P. Hanson. Plaque morphology of Newcastle Disease Virus as influenced by cell type and environmental factors. Am. J. Vet. Res. 29:883-895, 1968.

18. Schloer, G. and R.P. Hanson. Relationship of plaque size and virulence for chickens of 14 representitive Newcastle Disease Virus strains. J. Virol. 2:40-47, 1968.

19. Songer, J.R. Influence of relative humidity on the survival of some airborne viruses. Appl. Microbiol. 15:35-42, 1967.

20. Tyler, M.E., and E.L. Shipe. Bacterial aerosol samplers. I. Development and evaluation of the all glass impinger. Appl. Microbiol. 7:337-349, 1959.

Figure Legends

1. Physical half life of aerosolized fluorescein tracer in the rotating drum under different conditions of temperature and relative humidity.

2. Biological half life of aerosolized Newcastle Disease Virus (LaSota strain) in the rotating drum under different conditions of temperature and relative humidity.

Relative Humidity	Time (Hours)	% of Fluorescein Remaining*				
		Temperature (°C.)				
		10°	15°	20°	25°	30°
Low (20% or 30%**)	1	82	96	77	90	92
	2	72	91	63	83	78
	3	63	84	55	73	73
	4	56	73	48	70	59
	5	52	72	42	60	56
	6	51	70	36	59	55
Medium (50%)	1	88	89	99	85	83
	2	84	65	80	72	70
	3	69	69	63	64	64
	4	70	59	58	59	61
	5	59	53	53	53	59
	6	57	52	40	49	58
High (80%)	1	92	80	95	87	87
	2	79	67	83	76	74
	3	72	62	71	71	64
	4	82	61	64	64	59
	5	61	56	60	57	49
	6	68	53	52	66	49

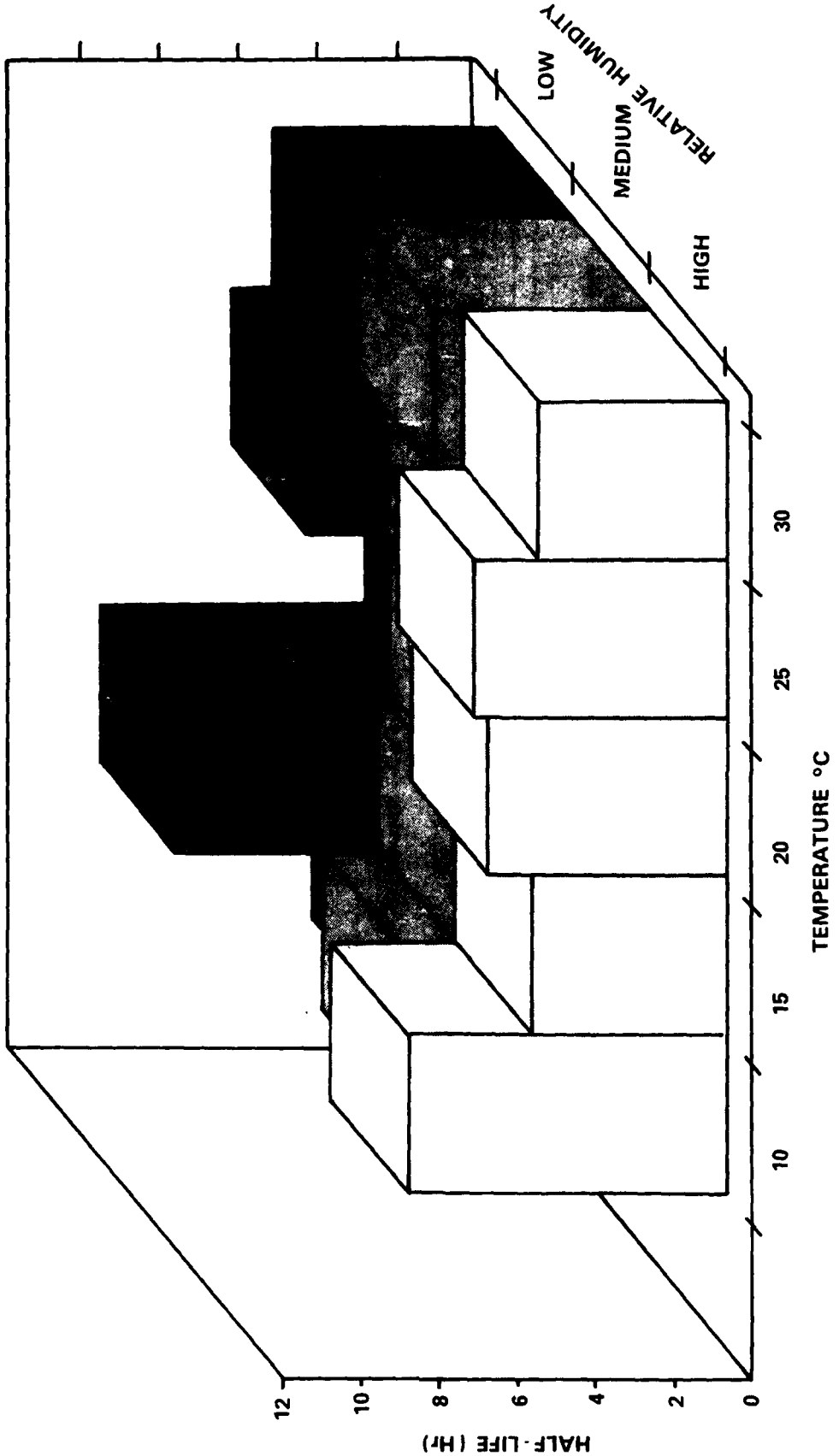
*Decay percentages shown are the means of 2 to 4 experiments, relative to 100% level at 0.25 hr.
**RH was 30% for 10° and 15°experiments, 20% for all others

Table 1. PHYSICAL DECAY OF FLUORESCEIN TRACER

Relative Humidity	Time (Hours)	% Virus Remaining*				
		Temperature (°C)				
		10°	15°	20°	25°	30°
Low (20% or 30%**)	1	82	78	85	79	67
	2	58	76	79	68	65
	3	67	67	78	47	56
	4	67	68	54	42	58
	5	68	64	33	42	66
	6	56	34	39	36	44
Medium (50%)	1	77	72	101	56	32
	2	80	47	51	37	29
	3	34	27	29	33	11
	4	60	27	39	18	14
	5	43	34	29	19	10
	6	26	18	18	13	11
High (80%)	1	101	91	78	62	38
	2	75	64	78	33	26
	3	69	25	24	41	24
	4	78	31	26	26	9
	5	35	39	26	14	3
	6	21	16	27	8	8

* Decay percentages shown are the means of 2 to 4 experiments, relative to 100% level at 0.25 hr.
**RH was 30% for 10°and 15° experiments, 20% for all others.

Table 2. BIOLOGICAL DECAY OF NDV


RELATIVE HUMIDITY
LOW
MEDIUM
HIGH
TEMPERATURE °C
0
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15
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25
30
HALF-LIFE (Hr)
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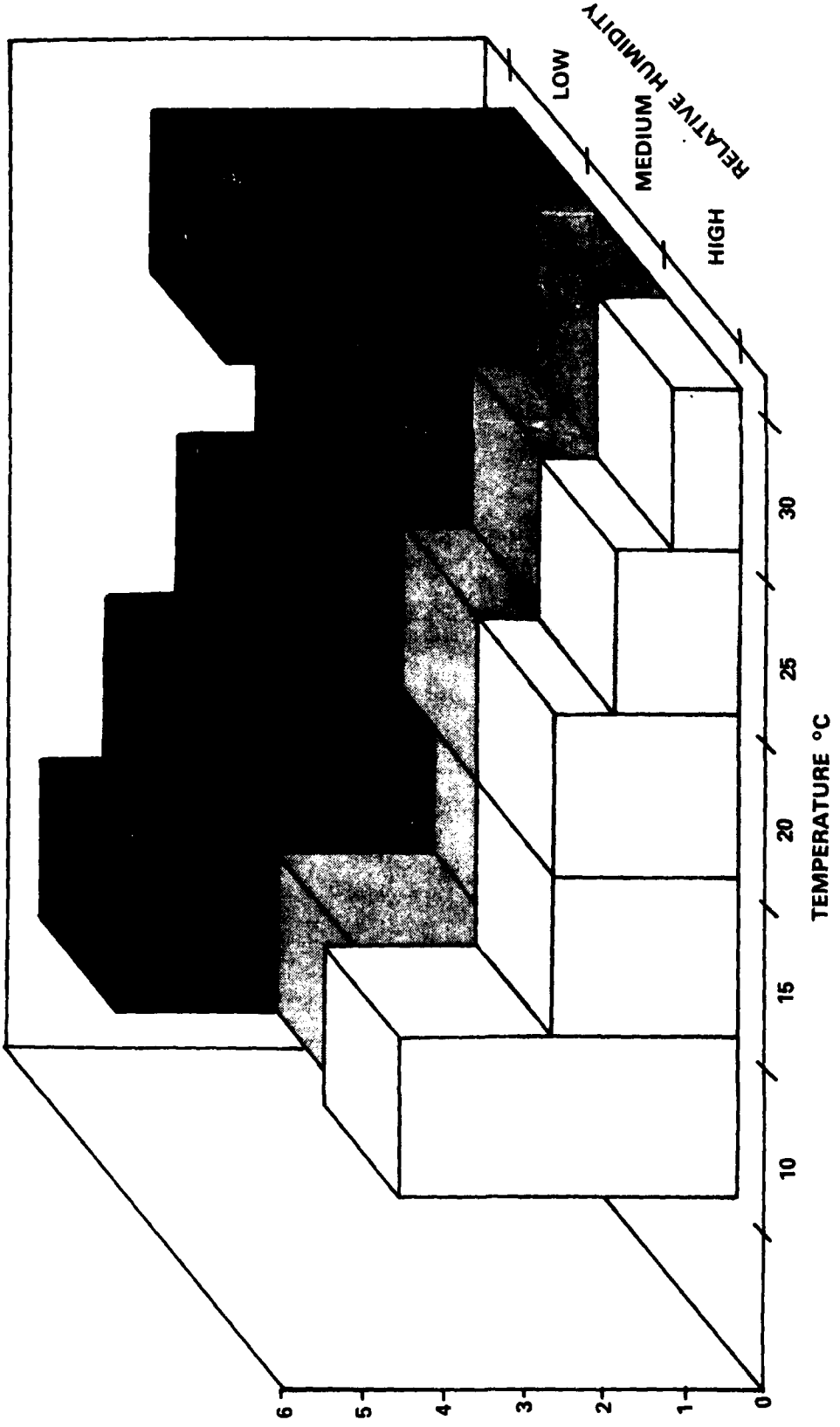
6
8
10
12

Figure 1


RELATIVE HUMIDITY
LOW
MEDIUM
HIGH
TEMPERATURE °C
10
15
20
25
30
HALF-LIFE (Hr)
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2
3
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Figure 2

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